Ecophysiology of a marine ultramicrobacterium

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It is generally assumed that the ocean represents a marine desert. Indeed, the ocean is responsible for only one-third of the world's primary production, whereas it represents two-thirds of its surface. However, the oceanic phytoplankton biomass equals only 0.05% of the terrestrial biomass. Therefore, per unit of biomass, oceanic ecosystems are far more productive than terrestrial systems and turnover rates of nutrients in the ocean are accordingly several hundred times higher. Interestingly enough, bacteria dominate the biomass of the oceans, partly because they out-compete the phytoplankton for nutrients. As a result, marine bacteria act as nutrient reservoirs, especially in oceanic regions where extremely low-nutrient conditions prevail. Marine bacteria therefore control the primary production. An overview of the different studies that have been performed on low-nutrient-adapted bacteria and of the ecological significance of marine bacteria is presented in Chapter 1.

Despite their importance, generally less than 1% of the bacteria present in seawater samples is culturable under laboratory conditions. Hence, little if anything is known about the bacteria that form the numerical majority of the marine bacterioplankton. While the average cell-volume of the dominant indigenous cells is approx. 0.05 μm^3, values for isolated marine bacteria are in most cases at least an order of magnitude higher. Hence, indigenous marine bacteria are often referred to as ultramicrobacteria. These small, freely floating cells are often viewed as dormant, unculturable forms of otherwise normal and well known bacterial groups. This concept is founded on the phenomenon that hitherto isolated marine bacteria form miniaturized, unculturable cells when starved. However, the existence of novel taxonomic groups has been proposed on the basis of genomic analysis of bacterioplankton. Furthermore, several lines of evidence indicate that these indigenous ultramicrobacteria are highly active under *in situ* conditions. The question as to whether or not these cells grow and what substrates they use is of considerable ecological importance. Aim of this study was to isolate and physiologically characterize representatives of these oligotrophic ultramicrobacteria.

A dilution technique for the cultivation of dominant marine bacteria has been developed in the course of this study. Details of this technique are presented in Chapter 2. The technique encompasses the inoculation of a small and known number of cells into unamended, sterilized seawater. The viability of inoculated bacteria can be estimated from their culturability in dilution tubes and statistical formulations for determining this value and the associated standard error have been derived. By using the dilution technique, between 2 and 60% of the cells present in seawater appears culturable. Such high values have never been obtained by previously employed laboratory cultivation techniques. High-resolution flow cytometry together with epifluorescence microscopy was applied to monitor development of the dilution cultures. At the highest dilutions of seawater, the majority of the cultures consisted of oligotrophic, low DNA-content ultramicrobacteria (Chapter 3). This is the bacterial cell-type that is of greatest ecological significance but has remained largely unculturable so far. After a starvation period of several months, several ultramicrobacterial strains were succesfully isolated by standard isolation techniques. The physiological changes that induced the culturability of these bacteria on nutrient-rich media remain subject to future investigation.

Strain RB2256, the isolate investigated, was obtained from Resurrection Bay, near Seward, Alaska, after a million-fold dilution of the original seawater sample. The organism

exhibits a mean cell-volume of
DNA content of 25 to 40% of
rRNA sequence, strain RB225
genus (Chapter 4). Phylogeneti
recently proposed genus of *Sph*
Sphingomonas [Flavobacterium
This phylogenetic cluster is pos
low-nutrient conditions. Two s
for strain RB2256. Results of
years after its isolation, strain
quantitative population analysi
unsuccessful due to the low nu

Strain RB2256 represen
realistic rates under oceanic lov
(Chapter 5). This ability is in pa
exhibiting a uniquely high speci
uptake. This permease is consti
small cell-size and low cellular
for cellular reproduction is min
protein dependent system. Alte
or phosphotransferase systems
suggesting that glucose is not a
is also too low to allow growt
alanine and glucose are both
simultaneously, even at relativel
substrate utilization is an intrins
were also utilized simultaneous
interesting aspects. Even under
immediately stored into polysac
in extraordinarily high endogeno
not readily serve as a resource

The starvation survival
marine bacteria investigated so f
of the cells and a decrease in c
rapidly in comparison to othe
specifically adapted to situations
that strain RB2256 is very well
the ocean, suggests that growth
ultramicrobacterium.